Diatom Detection in Drowning Investigations and Environmental Analysis –

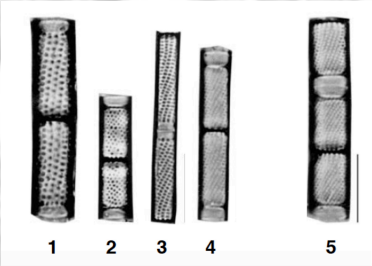
Development of a highly sensitive species-specific PCR Assay

Thomas Franks & Wera M Schmerer

Wolverhampton School of Science, Natural Sciences RG, University of Wolverhampton, UK, W.Schmerer@wlv.ac.uk

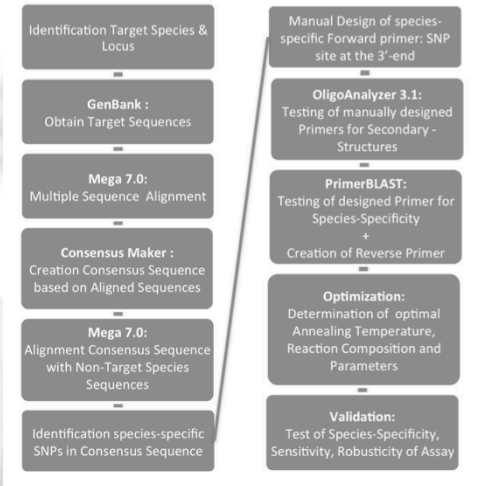
Diatoms are unicellular microorganisms which are commonly found in bodies of water in a multitude of different species. In cases of drowning, where the live victim breathes water when submerged, the diatoms contained enter the alveolar system and by way of the blood stream are transported into other organs such as bone marrow, kidney and brain (e.g. Incze 1942, 1951).
Presence or absence of diatoms in these organs can assist in distinguishing between actual drowning cases and post-mortem deposition of a corpse in a body of water (Peabody 1977), in which case diatoms would merely be found passively washed into the lungs.
Since the community of diatom species encountered is specific to the individual body of water, identification of the species present in the deceased can assist in distinguishing between the original site of the drowning and a secondary aquatic deposition site after the victim drowned elsewhere (e.g. Ludes et al 1999).
The composition of the diatom community in any given body of water is influenced by environmental factors, culminating in the quality of the water source. Consequently, analysis of the diatom community - in terms of species present (Naumann 1931) and relative ratio of the species composition (Zelinka and Marvan 1961) – can be utilized for the assessment of water quality (Kelly et al 1998).

Each of these approaches is based on the determination of diatom species represented. Traditionally, this assessment is accomplished by microscopic species determination based on morphological characteristics (e.g. Cox EJ 1997, Hürlimann & Niederhauser 2007).
Alternatively, diatom species can be identified utilizing species-specific targeted DNA analysis (e.g. Creach et al 2006). The presentation here features the early stages of the development of a new PCR-based assay for the species-specific detection of minute quantities of diatom DNA in aquatic samples.



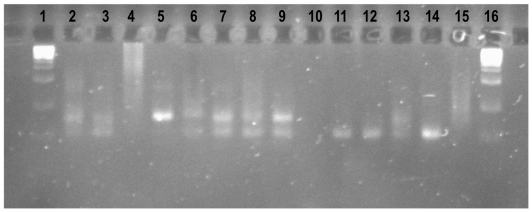
1-4 Aulacoseira granulata (EHRENBERG) SIMONSEN
5 Aulacoseira ambigua (GRUNOW) SIMONSEN

We began our own assay development with the design of primers targeting a section of the 18S rRNA gene of the genus *Aulacoseira, following a* previously established protocol for the design of species-specific primers (Halford et al 2017, 2018).



Sequences were obtained from Genbank (www.ncbi.nlm.nih.gov/genbank/). and aligned using the ClustalW multiple alignment tool (Thompson et al. 1994), provided by the Mega7.0 sequence analysis software (Kumar et al. 2016). Based on the resulting alignment, a consensus sequence was generated using Consensus Maker (www.hiv.lanl.gov/content/sequence/CONSENSUS/consensus.html).
The consensus sequence obtained was aligned with 18S rRNA gene sequences of related (other diatom species), sympatric and evolutionary distant species. The forward primer was then manually designed within highly variable regions and checked for secondary structures using the Integrated DNA Technologies (IDT) OligoAnalyzer 3.1 online tool (Owczarzy et al. 2008). The Primer-Blast online tool (Ye et al. 2012) was then utilized to test the species-specificity of the forward primers and to design appropriate reverse primers. To be viable for analysis of trace amounts of potentially degraded DNA, the focus was on sort amplicon sizes.
Three primer pairs (70, 88 and 93bp amplicon) were selected for synthesis, testing and validation in PCR amplifications of target-and non-target species samples. Further tests included the amplification of extracts derived from water samples collected in the field .

The primer pair targeting a 70bp amplicon proved to be the most viable of the three primer pair designs tested, in both amplification of samples confirmed to contain the species, as well as field water samples, while no amplification of non-species controls was detected (Franks 2018),
Based on and encouraged by the outcomes of this pilot study, the goal of future research would be the development of a multiplex approach targeting relevant indicator species for both, the characterization of a body of water with regards to water quality to be utilized in environmental analysis, as well as the distinction between different bodies of water for application in forensic investigation of drowning cases.



Amplification products from the tested 70bp amplicon primer pair. 1: Size Standard. 2, 3: Target Species Positive Control 1 and 2. 4, 9: Confirmed species-positive Water Samples. 5-8: Field Water Samples, 10-14: Non-species Control Samples. 15: no Template Control. 16: Size Standard.

Cox EJ (1997) The Identification of Freshwater Diatoms from Live Material. Chapman & Hall, London. **Creach V, Ernst A, Sabbe K, B. Vanelslander B, Vyverman W, Stal LJ (2006)** J Phycol 42: 1142-1154. **Franks T (2018**) BSc Thesis, University of Wolverhampton. **Halford CM, Jones KJ, Hill DJ, Schmerer WM (2018)**. InPractice 99. www.cieem.net. **Halford CM, Moreton-Jones KM, Hill DJ, Schmerer WM (2017)** Poster presentation. Mammal Society 63rd Spring Conference & AGM, 31 March 2017, Cambridge, UK. **Hürlimann J & Niederhauser P (2007)** Methoden zur Untersuchung und Beurteilung der Fliessgewaesser. BAFU, Switzerland. **Incze G (1942)** Zentralbl Allg Patbol Anat 79: 176. **Incze G (1951)** Actll Morpbol Hung 1: 421-430. **Kelly MG, Cazaubon A, Coring E et al. (1998)** Recommendations for the routine sampling of diatoms for water quality assessments in Europe. J Appl Phycol 10: 215-224. **Kumar S, Stecher G, Tamura K (2016)** Mol Biol Evol, 33(7): 1870-1874. **Ludes B, Coste M, North N, Doray S, Tracqui A, Kintz P (1999)** Int J Legal Med 112: 163-166. **Naumann E (1931)** Limnologische Terminologie. In: Abderhalden (ed.) Handbuch der biologischen Arbeitsmethoden, Abt. IX, Teil 8. Schwarzenberg, Berlin. **Owczarzy R, Tataurov AV, Wu Y, Manthey JA, McQuisten KA, Almabrazi HG, Pedersen KF, Lin Y, et al (2008)** Nucl Acids Res 36(Web Server Issue): W163-W169. **Peabody AJ (1977)** J Forensic Sci So 17:81-87. **Thompson JD, Higgins DG & Gibson TJ (1994)** Nucl Acids Res 22(22): 4673-4680. **Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL (2012)** BMC Bioinformatics, 13(1): 134. **Zelinka M & Marvan P (1961)** Arch Hydrobiol 57: 389-407.Background: Haeckel E (1900) Kunstformen der Natur. Verlag des Bibliographischen Instituts